II. REMARKS

Formal Matters

Claims 7-14, 17-20, 25-30, and 32-35 are pending after entry of the above-noted amendments.

Claims 12-16 and 32-37 were examined and were rejected. Claims 7-11, 17-20, and 25-30 were withdrawn from consideration.

Claim 12 is amended. The amendment to claim 12 was made solely in the interest of expediting prosecution, and is not to be construed as acquiescence to any objection or rejection of any claim. No new matter is added by the amendment to claim 12.

Claims 15, 16, 36, and 37 are canceled without prejudice to renewal, without intent to acquiesce to any rejection, and without intent to surrender any subject matter encompassed by the canceled claims. Applicants expressly reserve the right to pursue any canceled subject matter in one or more continuation and/or divisional applications.

Applicants respectfully request reconsideration of the application in view of the remarks made herein.

Examiner Interview

The undersigned Applicants' representative thanks Examiner Michael Pak and Examiner Gary Nickol for the courtesy of a telephonic interview which took place on June 4, 2009, and which was attended by Examiners Pak and Nickol, inventor Yadong Huang, and Applicants' representative Paula A. Borden.

During the interview, the rejections under 35 U.S.C. § 112, second paragraph, and §102, were discussed. The amendments to the claims reflect the discussions which took place during the interview.

Rejection under 35 U.S.C. §112, second paragraph

Claims 12-16 and 32-37 were rejected under 35 U.S.C. §112, second paragraph, as allegedly indefinite.

The Office Action stated that "amino acids 244-260 of apoE" is ambiguous and unclear, and stated that the metes and bounds are unclear. Applicants respectfully traverse the rejection.

Requirements under 35 U.S.C.§112, second paragraph

All that is required under 35 U.S.C. §112, second paragraph, is that the claims set out the subject matter **with a reasonable degree of clarity and particularity**. As set forth in MPEP §2173.02, in reviewing a claim for compliance with 35 U.S.C. §112, second paragraph, the examiner must consider the claim as a whole to determine whether the claim apprises one of ordinary skill in the art of its scope, in other words, whether the scope of the claim is clear to a person of ordinary skill in the relevant art.

As set forth in MPEP §2173.02, definiteness of claim language must be analyzed in light of:

a) the content of the disclosure of the patent application;

b) the teachings of the prior art; and

c) the claim interpretation that would be given by one possessing the ordinary level of skill in the art at the time the invention was made.

As discussed during the June 4, 2009 telephone interview, those skilled in the art know what is meant by "amino acids 244-260 of apoE" at least because:

1) The amino acid sequences of apoE were well known as of the November 3, 2000 priority date;
2) The amino acid sequences of apoE from various species are conserved;
3) Lipid binding properties of the C-terminal domain and receptor-binding properties of the N-terminal domain of apoE were known as of the November 3, 2000 priority date.

Amino acid sequences of apoE were known as of the November 3, 2000 priority date.

As noted in the instant specification, apoE sequences (amino acid sequences, nucleotide sequences) were well known in the art as of the priority date of the instant application. For example, the instant specification states:

> The sequence of the mouse apoE gene is found under Genbank accession number D00466. Various primate apoE gene sequences are found under GenBank accession numbers AF200508, AF200507, AF200506, and AH009953 (*Hylobates lar*, or gibbon); AH009952, AF200503, AF200504, and AF200505 (*Pongo pygmaeus*, or orangutan); AH009951, AF200500, AG200501, and AF200502 (*Gorilla gorilla*); AH009950, AF200497, AF200498, AF200499 (*Pan troglodytes*, or chimpanzee). Any apoE-encoding sequence can be modified to encode a carboxyl-terminal truncated apoE polypeptide as described above.

Specification, paragraph 0049

Those skilled in the art were well aware of various apoE sequences and would have known what amino acids correspond to amino acids 244-260.

The amino acid sequences of apoE of various species are conserved.

It was known as of the November 3, 2000 priority date that amino acid sequences of apoE are conserved. Weisgraber ((1994) *Adv. Protein Chem.* 45:249-302; "Weisgraber 1994") provides a figure (Figure 1 on page 252 of Weisgraber 1994), which provides an alignment of amino acid sequences of apoE from a variety of species. A copy of Weisgraber 1994 is provided herewith.

As noted in Weisgraber 1994, the carboxyl-terminal, lipid-binding domain is apoE is highly conserved.

> Across species the carboxyl-terminal region of apoE up to approximately residue 288 in the human sequence is highly conserved (Fig. 1). Studies to determine the carboxyl-terminal regions of apoE responsible for lipid binding and tetramer formation have been performed using three carboxyl-terminal truncations (Westerlund and Weisgraber, 1993). As shown in Fig. 19, the carboxyl terminus beyond position 191

Weisgraber 1994, page 44.

The C-terminal domain of apoE was known as of the November 3, 2000 priority date to be hydrophobic and lipid binding.

The overall structure of apoE was well known as of the November 3, 2000 priority date. It was known that apoE is composed of two major structural and functional domains: 1) a 22-kDa N-terminal domain that contains the receptor binding region; and 2) an approximately 10-kDa C-terminal domain that has a high affinity for lipid and is responsible for lipoprotein binding. See, e.g., Weisgraber 1994 (above) and Weisgraber and Mahley ((1996) *FASEB J.* 10:1485; "Weisgraber 1996").

For example, Weisgraber 1994 states:

As noted in Weisgraber 1994, the carboxyl-terminal, lipid-binding domain is apoE is highly conserved.

> Across species the carboxyl-terminal region of apoE up to approximately residue 288 in the human sequence is highly conserved (Fig. 1). Studies to determine the carboxyl-terminal regions of apoE responsible for lipid binding and tetramer formation have been performed using three carboxyl-terminal truncations (Westerlund and Weisgraber, 1993). As shown in Fig. 19, the carboxyl terminus beyond position 191

Weisgraber 1994, page 44.

As another example, Weisgraber 1996 states:

> different functions of apoE (21, 22). The amino-terminal domain (residues 1–191) contains the lipoprotein receptor binding region (residues 136–150) (23), and the carboxyl-terminal domain (residues 216–299) contains the major lipoprotein (lipid) binding determinants (Fig. 2) (24). The structure of the amino-terminal domain, deter-

and

> depicted as a series of helices in Fig. 2. Studies involving carboxyl-terminal truncations demonstrated that residues 244–272 contain elements critical for lipoprotein (lipid) binding and the lipoprotein preferences of the isoforms (26).

Weisgraber 1996, page 1487

These features of apoE are reviewed in Mahley and Huang (1999) *Curr. Opin. Lipidol.* 10:207.

Finally, Applicants invite the examiner to review the file history of the parent application, 10/033,526, which issued as U.S. Patent No. 6,787,519. In the parent application, claims reciting "neurotoxic carboxyl-terminal truncated form of apoE" and "wherein the carboxyl-terminal truncated apoE comprises amino acids 244-260 of apoE" issued.

Conclusion as to the rejection under 35 U.S.C. §112, second paragraph

Applicants submit that the rejection of claims 12-16 and 32-37 under 35 U.S.C. §112, second paragraph, has been adequately addressed in view of the remarks set forth above. The Examiner is thus respectfully requested to withdraw the rejection.

Rejections under 35 U.S.C. §102(b)

Claims 12-16 and 32-37 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by Crutcher and Harmony (WO 98/01101). Claims 6-12 and 32-37 were rejected under 35 U.S.C. §102(b) as allegedly anticipated by Tolar et al. ((1999) *J. Neurosci.* 19:7100; "Tolar").

As discussed during the June 4, 2009 telephone interview, the inventors listed on WO 98/01101 and the co-authors listed on Tolar are from the same group. WO 98/01101 lists Keith A. Crutcher as an inventor. Tolar lists Keith A. Crutcher as the senior author. Also as discussed during the June 4, 2009 telephone interview, the N-terminal fragment discussed in WO 98/01101 and in Tolar is a 22-kDa thrombin cleavage product. This 22-kDa thrombin cleavage product is an artificial product (e.g., is not normally generated *in vivo*) and has been found not to be neurotoxic.

Claims 12-16 and 32-37; WO 98/01101

As noted above, and as explained previously, e.g., in the amendment, filed on November 6, 2008 and responsive to the Office Action dated August 7, 2008, WO 98/01101 neither discloses nor suggests a screening method involving identifying agents that reduce formation of a neurotoxic carboxyl-truncated apoE fragment.

WO 98/01101 focuses on a region of apoE from **amino acids 130-169**. WO 98/01101, page 11, lines 30-31. WO 98/01101 states that the 22 kD thrombin cleavage fragment of apoE is neurotoxic, and can be used to assess the efficacy of test compound. WO 98/01101, page 13, lines 30-34. WO 98/01101 discusses an assay to test for inhibition of neurotoxicity of the 22 kD fragment, which assay involves treating neuronal cells *in vitro*

with a test agent and 22 kD fragment, and determining the effect of the test agent on inhibition of neurotoxicity of the 22 kD fragment. WO 98/01101, Example 2, page 14, lines 16-31. Nowhere does WO 98/01101 disclose or suggest inhibition of formation of a neurotoxic carboxyl-terminal truncated apoE fragment, **wherein the neurotoxic carboxyl-terminal truncated apoE polypeptide comprises amino acids 244-260 of apoE**.

However, there is evidence in the instant application that the 22 kD thrombin cleavage fragment of apoE is not neurotoxic. Instead, the instant application provides evidence that carboxyl-terminal truncated apoE fragments that lack amino acids 244-260 are not neurotoxic. Specification, paragraphs 00215 and 00216; and Figure 3B. The 22 kD thrombin cleavage fragment of apoE consists of amino acids 1-191 of apoE. Thus, the 22 kD thrombin cleavage fragment lacks amino acids 244-260 identified in the instant application as essential for neurotoxicity. These data indicate that, in contrast to the assertion in WO 98/01101, the presence of amino acids 130-169 is not critical for neurotoxicity.

In addition, there is evidence in the art that peptides corresponding to the apoE receptor binding site are neuroprotective, not neurotoxic. See, *e.g.*, Aono et al. ((2003) *Neurosci.* 116:437-445; "Aono"; a copy of which was previously provided). Aono reports that a peptide derived from the receptor binding region of apoE (**residues 133-149**) completely suppressed neuronal cell death and calcium influx associated with N-methyl-D-aspartate exposure, and that this peptide is thus neuroprotective. Aono, Abstract. Aono discusses the contrast in the observation with the report in Tolar that the 22 kD apoE thrombin cleavage fragment is neurotoxic, states that Tolar used a peptide comprised of tandem repeats of residues 141-149, and showed neuronal cell death upon exposure to this peptide. Aono et al., page 444, column 1, second paragraph. A peptide containing tandem repeats of residues 141-149 is an artificial construct made in the laboratory, and does not exist *in vivo*. Aono further states that the tandem repeat may not be a biologically relevant model of the intact apoE protein. Aono et al., page 444, column 1, second paragraph.

Because WO 98/01101 neither discloses nor suggests a screening method involving identifying agents that reduce formation of a neurotoxic carboxyl-truncated apoE fragment, **wherein the neurotoxic carboxyl-terminal truncated apoE polypeptide comprises amino acids 244-260 of apoE**, WO 08/01101 cannot anticipate any of claims 12-16 and 32-37.

Claims 15 and 16

Claims 15 and 16 are cancelled without prejudice to renewal, thereby rendering any rejection of claims 15 and 16 moot.

Claims 6-12 and 32-37

Applicants note that claim 6 was previously cancelled, and that claims 7-11 were withdrawn from consideration. Applicants request clarification as to which claims are included in this rejection. In the meantime, Applicants will respond to the rejection as it might apply to claims 12-16 and 32-37.

The Office Action stated that Tolar discloses a method of using a protease inhibitor cocktail to attenuate the production of neurotoxic apoE4 fragments in dissociated chick sympathetic neurons. The Office Action stated that Tolar discloses that protease inhibition reduces the formation of neurotoxic apoE fragments. Applicants respectfully traverse the rejection.

Tolar neither discloses nor suggests a screening method involving identifying agents that reduce formation of a neurotoxic carboxyl-truncated apoE fragment.

Tolar discusses the effect of a **22 kD apoE fragment** and a tandem repeat of amino acids **141-149** of apoE. Tolar, Abstract; page 7102, column 1, first paragraph under "Results." Tolar states that exposure of neurons to full-length apoE resulted in the appearance of lower molecular weight fragments of apoE, including a 22 kD fragment, "which most likely represents the major N-terminal fragment of apoE." Tolar, page 7102, column 1, first paragraph under "Results." As discussed above, the 22 kD major N-terminal fragment of apoE lacks amino acids 244-260 which were shown in the instant specification to be important for neurotoxicity of carboxyl-terminal truncated apoE. Tolar also states that the artificial peptide consisting of a tandem repeat of amino acids 141-149 is neurotoxic. Tolar, Abstract; and page 7102, column 1, first paragraph under "Results."

Nowhere does Tolar disclose or suggest inhibition of formation of a neurotoxic carboxyl-terminal truncated apoE fragment, **wherein the neurotoxic carboxyl-terminal truncated apoE polypeptide comprises amino acids 244-260 of apoE**.

Because Tolar neither discloses nor suggests a screening method involving identifying agents that reduce formation of a neurotoxic carboxyl-truncated apoE fragment, **wherein the neurotoxic carboxyl-terminal truncated apoE polypeptide comprises amino acids 244-260 of apoE,** Tolar cannot anticipate any of claims 12-16 and 32-37.

As discussed above, and as discussed during the June 4, 2009 telephone interview, the 22 kDa fragment of Tolar has been reported not to be neurotoxic. For example, as discussed above, Aono reported that a peptide derived from the receptor binding region of apoE (**residues 133-149**) completely suppressed neuronal cell death and calcium influx associated with N-methyl-D-aspartate exposure, and that this peptide is thus neuroprotective.

Aono discusses the contrast in the observation with the report in Tolar that the 22 kD apoE thrombin cleavage fragment is neurotoxic, states that Tolar used a peptide comprised of tandem repeats of residues 141-149, and showed neuronal cell death upon exposure to this peptide. Aono et al., page 444, column 1, second paragraph. A peptide containing tandem repeats of residues 141-149 is an artificial construct made in the laboratory, and does not exist *in vivo*. Aono further states that the tandem repeat may not be a biologically relevant model of the intact apoE protein. Aono et al., page 444, column 1, second paragraph.

The following references also report neuroprotective effects of peptides derived from the receptor-binding, N-terminal domain of apoE:

Li et al. ((2006) *J. Pharmacol. Exp. Ther.* 318:956; "Li") reports the effect of an apoE-derived peptide that includes residues **133-149** of apoE in ameliorating the pathology of experimental autoimmune encephalitis in a mouse model of multiple sclerosis

Lynch et al. (2005) *Exp. Neurol.* 192:109; McAdoo et al. (2005) *Neurosci. Lett.* 381:305

McAdoo et al. (2005) *Neurosci. Lett.* 381:305.

Gay et al. ((2006) *J. Pharmacol. Exp. Ther.* 316:835; "Gay") notes the neuroprotective effects reported by Lynch and by McAdoo, and report that two apoE-derived peptides – one consisting of residues **133-149** and the other consisting of residues **141-148** – disrupt nAChR signaling. Gay postulates that the disruption explains the neuroprotective effects of these peptides. Gay, page 841, column 2.

Conclusion as to the rejections under 35 U.S.C. §102(b)

Applicants submit that the rejections of claims 12-16 and 32-37 under 35 U.S.C. §102(b) have been adequately addressed in view of the remarks set forth above. The Examiner is thus respectfully requested to withdraw the rejections.

III. CONCLUSION

Applicants submit that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number GLAD-217CON.

Respectfully submitted,
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Date: 20 August 2009

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